

Claims

1. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme, wherein one or more amino acid residues in a region of 349-377 amino acid of water-soluble PQQGDH derived from *Acinetobacter calcoaceticus* is replaced with other amino acid residues and has an inhibition constant (K_{si}) of 200 mM or more.
2. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein Met365 of the amino acid sequence defined in SEQ ID NO: 1 is replaced with another amino acid, and has a K_{si} value of 200 mM or more.
3. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein Met365 of the amino acid sequence defined in SEQ ID NO: 1 is replaced with tryptophan or phenylalanine.
4. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein Thr366 of the amino acid sequence defined in SEQ ID NO: 1 is replaced with another amino acid, and has a K_{si} value 200 mM or more.
5. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein Thr366 of the amino acid sequence defined in SEQ ID NO: 1 is replaced with aspartic acid, lysine, isoleucine, or asparagines.
6. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein Tyr367 of the amino acid sequence defined in SEQ ID NO: 1 is replaced with another amino acid, and has a K_{si} value of 200 mM or more.

7. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein Tyr367 of the amino acid sequence defined in SEQ ID NO: 1 is replaced with aspartic acid.
8. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein Ile368 of the amino acid sequence defined in SEQ ID NO: 1 is replaced with another amino acid, and has a K_{si} value of 200 mM or more.
9. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein Ile368 of the amino acid sequence defined in SEQ ID NO: 1 is replaced with asparagine.
10. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein Cys369 of the amino acid sequence defined in SEQ ID NO: 1 is replaced with another amino acid and has a K_{si} value of 200 mM or more.
11. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein Cys369 of the amino acid sequence defined in SEQ ID NO: 1 is replaced with arginine.
12. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein Ala374 of the amino acid sequence defined in SEQ ID NO: 1 is replaced with another amino acid, and has a K_{si} value of 200 mM or more.
13. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein Ala374 of the

amino acid sequence defined in SEQ ID NO: 1 is replaced with proline.

14. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein one of the amino acids in 349-377 region of the amino acid sequence defined in SEQ ID NO: 1 and Asp167 are replaced with other amino acids.

15. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein an amino acid residue selected from the group consisting of Met365, Thr366, Tyr367, Ile368, Cys369, and Ala374 of the amino acid sequence defined in SEQ ID NO: 1 is replaced with another amino acid and wherein Asp167 is replaced with another amino acid.

16. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein an amino acid residue selected from the group consisting of Met365, Thr366, Tyr367, Ile368, Cys369, and Ala374 of the amino acid sequence defined in SEQ ID NO: 1 is replaced with another amino acid and wherein Asp167 is replaced with glutamic acid.

17. A modified glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme wherein Thr366 of the amino acid sequence defined in SEQ ID NO: 1 is replaced with aspartic acid, lysine, isoleucine, or asparagine, and wherein Asp167 is replaced with glutamic acid.

18. A glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme comprising the following amino acid sequence:
Cys Gly Glu Xaa Thr Tyr Ile
wherein Xaa is Met or Trp.

19. A glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme comprising the following amino acid sequence:
Gly Glu Met Xaa Tyr Ile Cys

wherein Xaa is Asp, Lys, Ile or Asn.

20. A glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme comprising the following amino acid sequence:
Glu Met Thr Asp Ile Cys Trp.

21. A glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme comprising the following amino acid sequence:
Met Thr Tyr Asp Cys Trp Pro.

22. A glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme comprising the following amino acid sequence:
Thr Tyr Ile Arg Trp Pro Thr.

23. A glucose dehydrogenase having pyrroloquinoline quinone as a coenzyme comprising the following amino acid sequence:
Pro Thr Val Pro Pro Ser Ser.

24. A gene encoding a modified glucose dehydrogenase as claimed in any one of claims 1 to 23.

25. A vector comprising the gene as claimed in claim 24.

26. A transformant comprising the gene as claimed in claim 24.

27. A transformant as claimed in claim 26 wherein the gene as claimed in claim 24 is integrated in its chromosome.

28. A method for preparing a water-soluble PQQGDH, comprising culturing the transformant as claimed in claim 27 and preparing water-soluble fraction from the cells of the transformant.

29. A glucose assay kit comprising the modified glucose dehydrogenase as claimed in any one of claims 1-23.

30. A glucose sensor comprising the modified glucose dehydrogenase as claimed in any one of claims 1-23.